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THE OPTICAL ROTATORY DISPERSION OF γ-LINKED OLIGO- AND POLYPEPTIDES OF GLUTAMIC ACID

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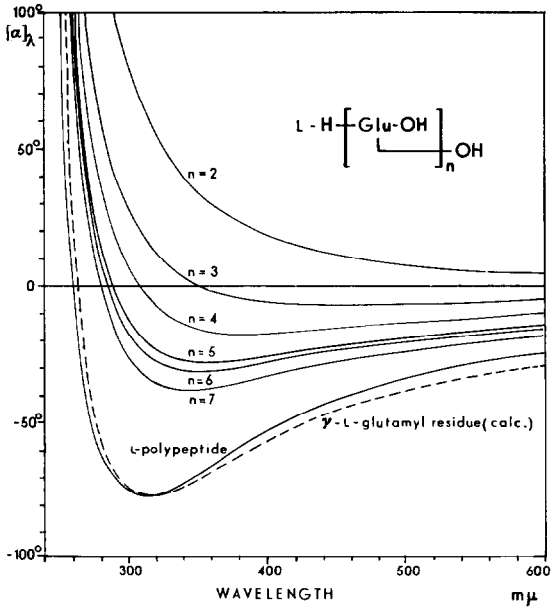
The secondary structure of native γ-poly-D-glutamic acid was studied recently by Rydon (1) through the measurement of optical rotatory dispersion. The ORD of the polypeptide in alkaline solution was found to be simple, conforming to a single-term Drude equation, whereas the rotation of the free acid (in aqueous solution) showed a complex dispersion, the description of which was accomplished by applying a modified Moffitt equation with $\lambda_0 = 197$ mμ. From these results Rydon concluded that un-ionised γ-poly-D-glutamic acid possesses a helical structure. He suggested two possible left handed helical models.

In order to determine the secondary structure of synthetic γ-polyglutamic acid (2), we have measured the ORD of both its antipodes in acid as well as in alkaline solution from 600 mμ to 250 mμ. The ORD of the synthetic and of the native γ-poly-D-glutamic acid has been found to be about the same, indicating them to possess the same secondary structure. The dispersion curves belonging to the two antipodes of the synthetic polypeptide are mirror images.

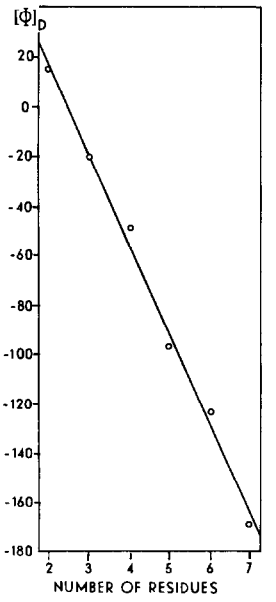
The synthesis (3) of a series of γ-linked oligopeptides derived from L-glutamic acid has given us the possibility of measuring their ORD, too. The rotations found in acid solution are shown in Fig.1. Already by the shapes of the ORD curves show that, starting from the seemingly plain dispersion curve of the dipeptide, the curves of the oligomers become gradually more complex and more similar to that of the polymer. No definite change, however, in the characteristic features of the ORD curves can be detected between the lower and the higher oligomers on the one hand, or between the oligomers and the polymer, on the other.

FIG. 1.



ORD Curves of γ-L-Glutamyl Oligopeptides and Polypeptide in Acid

FIG. 2.



Molar Rotations (at 558 mμ) of γ-Glutamyl Oligopeptides

The molar rotations of the oligomers, plotted against the number of residues (cf. 4), fall on a straight line at every wavelength examined. (The plot of $[\phi]_D$ values can be seen in Fig.2.) From the slopes of these straight lines the total ORD curve due to the contribution to the molar rotation by a single internal γ-glutamyl residue can be calculated. This curve (dotted line in Fig.1.) coincides surprisingly well with that of the γ-polyglutamic acid (full line).

From these results we must conclude that neither the higher oligomers nor the polymer possess any secondary structure different from that of the lower oligomers, and, in consequence, all of them are in random configuration not only in alkaline, but also in acid solution. The complex rotatory dispersion shown by the un-ionised γ-polyglutamic acid does not arise from a highly ordered helical structure of the polymeric molecule, but it is an inherent property of the internal γ-glutamyl residue which manifests itself already in the ORD of small peptides.

As to Rydon's conclusion concerning the helical structure of γ-polyglutamic acid, it may be remarked that the application of the Moffitt equation with a λ_o different from the original 212 mμ (5), and in the case of a polypeptide having a primary structure quite different from that of α-polypeptides, should be misleading. Moreover, the mere fact that ORD data fit the Moffitt equation should not, in any instance, be considered as the sign of helical structure (cf. 6).

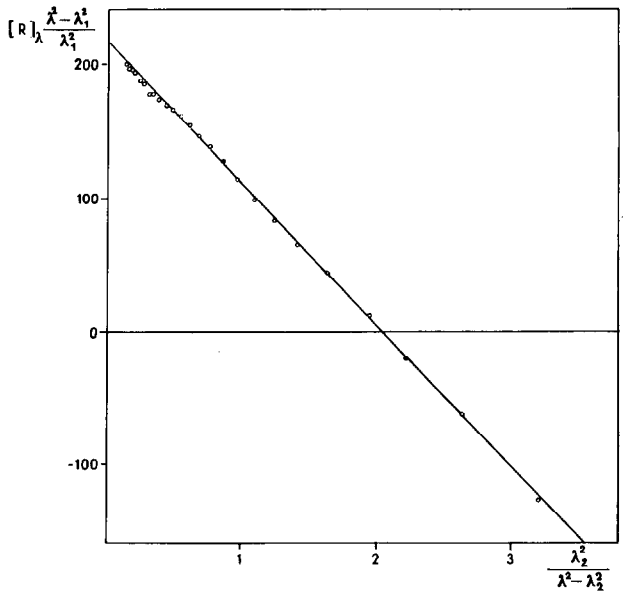
It seems more useful to apply a two-term Drude equation (cf. 7) for the description of the complex dispersion of both

the γ-polyglutamic acid and the oligomers. We have found the ORD of all of the substances mentioned above to conform, in a quite satisfactory agreement, to the following equation:

$$[\alpha] = \frac{a_1\lambda_1^2}{\lambda^2-\lambda_1^2} + \frac{a_2\lambda_2^2}{\lambda^2-\lambda_2^2} \quad \text{, where} \quad \begin{matrix}\lambda_1 = 192\ m\mu \\ \lambda_2 = 213\ m\mu\end{matrix} \qquad \text{(Equ.1.)}$$

The $\underline{a}_1$ and $\underline{a}_2$ constants were obtained graphically (7) from experimental rotations. (The two-term Drude plot of γ-poly-D-glutamic acid is shown in Fig.3.) The values of $\underline{a}_1$ and $\underline{a}_2$ indicate that the molar rotations of the oligopeptides are composed additively from the contributions by the individual chromophores, namely the CONH and COOH groups, attached to

FIG. 3.



Two-term Drude Plot of γ-Poly-D-glutamic Acid

the asymmetric centres (cf. 8). It can be supposed that the first term of the above equation expresses the contribution of the amide groups, whereas the second arises from the collective contribution of the α-carboxyl and the amide groups.* In accordance with these considerations, the $\underline{a}_1$ and $\underline{a}_2$ constants, for all the investigated γ-oligopeptides of L-glutamic acid, can be obtained from the following expressions, $\underline{n}$ being the number of glutamic acid residues and $\underline{M}$ the molecular weight:

$$a_1 = -\frac{100}{M}\,[1120\,(n-1)] \qquad \text{(Equ.2.)}$$

$$a_2 = +\frac{100}{M}\,[350\,n + 317\,(n-1)] \qquad \text{(Equ.3.)}$$

The difference between the corresponding values of $\underline{a}_1$ and $\underline{a}_2$, obtained from the two-term Drude plots and from the above expressions, respectively, is less than 5% (Table I). This agreement should be considered as quite satisfactory.

By this method of analysis not only the contributions of the different asymmetric parts of the molecules (cf. 8), but also the effect of the dichroic properties of the individual chromophores could be estimated.

The ORD study of other ω-linked derivatives of α-aminodicarboxylic acids is in progress.

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* The circular dichroic spectra of randomly disordered α-L-polypeptides (9) show a strong negative dichroic band due to the $\pi \rightarrow \pi^*$ amide transition with a peak at 191 mμ, and a weakly positive dichroic band near 220 mμ which is most probably the $n \rightarrow \pi^*$ transition of the amide group. The carboxyl group has -according to the ORD of β-unsubstituted amino acids (10)- a positive Cotton effect centered at about 210-215 mμ, belonging probably to the optically active $n \rightarrow \pi^*$ transition band.

TABLE I

Rotatory Constants of γ-Glutamyl Oligopeptides in Acid

	Calculated from equations 2. and 3.			Determined from two-term Drude plots		
	a_1	a_2	a_1/a_2	a_1	a_2	a_1/a_2
Dipeptide (L)	-406	+358	-1,104	-421	+371	-1,135
Tripeptide (L)	-552	+409	-1,350	-520	+384	-1,355
Tetrapeptide (L)	-630	+436	-1,445	-614	+425	-1,445
Pentapeptide (L)	-676	+452	-1,495	-704	+466	-1,510
Hexapeptide (L)	-708	+462	-1,532	-684	+447	-1,528
Heptapeptide (L)	-726	+469	-1,550	-752	+485	-1,550
Polypeptide (D)	+868	-516	-1,660	+766	-447	-1,714

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